OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

Mag-Bind® Blood & Tissue DNA HDQ 96 Kit

M6399-00	1 x 96 preps
M6399-01	4 x 96 preps

April 2015

For research use only.Not intended for diagnostic testing.

Mag-Bind® Blood & Tissue DNA HDQ 96 Kit

Table of Contents

Introduction and Overview....2
Kit Contents....3
Storage and Stability....3
Magnetic Separation Devices and Plasticware....3
Preparing Reagents....4
Mag-Bind® Blood Protocol (250 µL)....5
Mag-Bind® Tissue Protocol....8
Mag-Bind® Cultured Cells Protocol....12
Mag-Bind® Saliva Protocol....16
Mag-Bind® Buccal Swabs Protocol....19
Mag-Bind® Tail Snips Protocol....23
Troubleshooting Guide....27
Ordering....28

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Introduction

The Mag-Bind® Blood & Tissue DNA HDQ 96 Kit is designed for rapid and reliable isolation of high-quality genomic DNA from 250 µL blood samples, tissue, saliva, buccal swabs, cultured cells, and mouse tail snips. Mag-Bind® Particles HDQ provide a quick magnetic response time reducing overall processing time. This system combines the reversible nucleic acid-binding properties of Mag-Bind® paramagnetic particles with the time-proven efficiency of Omega Bio-tek's DNA isolation system to provide a fast and convenient method to isolated DNA from a variety of samples. Utilizing paramagnetic particles provides high-quality DNA that is suitable for direct use in most downstream applications, such as amplification and enzymatic reactions.

Overview

If using the Mag-Bind® Blood & Tissue DNA HDQ 96 Kit for the first time, please read this booklet in its entirety to become familiar with the procedures. Samples are lysed in buffer systems that are tailored to each type of starting material. DNA is isolated from the lysates by binding to Mag-Bind® Particles' surfaces. The paramagnetic particles are separated from the lysates by using a magnetic separation device. After a few rapid wash steps to remove trace contaminants, DNA is eluted in Elution Buffer.

New in this Edition:

- M6399 Mag-Bind® Blood DNA HDQ 96 Kit is now Mag-Bind® Blood & Tissue DNA HDQ 96 Kit.
- Five additional protocols for Tissue, Cultured Cells, Saliva, Buccal Swabs, and Mouse Tail Snips have been included.
- TL Buffer has been added to the kit for the Tissue Protocols.
- The volume of Elution Buffer has been increased.

Kit Contents

Product	M6399-00	M6399-01
Preps	1 x 96	4 x 96
Mag-Bind® Particles HDQ	2.2 mL	9 mL
AL Buffer	35 mL	125 mL
TL Buffer	30 mL	120 mL
HDQ Binding Buffer	10 mL	40 mL
VHB Buffer	55 mL	220 mL
SPM Wash Buffer	30 mL	120 mL
Proteinase K Solution	2.2 mL	9 mL
Elution Buffer	50 mL	200 mL
User Manual	✓	✓

Storage and Stability

All of the Mag-Bind® Blood & Tissue DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Particles HDQ should be stored at 2-8°C for long-term use. Proteinase K Solution can be stored at room temperature for up to 12 months. For long-term storage, store Proteinase K Solution at 2-8°C.

Magnetic Stand and Plasticware

While many brands of magnetic separation devices are compatible with the Mag-Bind® Blood and Tissue DNA 96 Kit, we recommend using AlpAqua's Magnum™ EX Universal Magnet Plate (Part# A000380) in conjunction with Nunc 2 mL DeepWell™ plates (Part# 278752). This combination provides quick magnetization times, only 1 minute for complete magnetization during wash steps and 5 minutes for lysate clearance steps. Please contact an Omega sales associate or customer service representative for ordering information (1-800-832-8896).

Regardless of the magnetic separation device selected, ensure the device is compatible with the plasticware necessary for this kit.

Preparing Reagents

1. Dilute SPM Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M6399-00	70 mL
M6399-01	280 mL

2. Prepare VHB Buffer as follows and store at room temperature.

Kit	100% Ethanol to be Added
M6399-00	70 mL
M6399-01	280 mL

3. Prepare HDQ Binding Buffer as follows and store at room temperature.

Kit	100% Isopropanol to be Added
M6399-00	40 mL
M6399-01	160 mL

4. Shake or vortex the Mag-Bind® Particles HDQ to fully resuspend the particles before use. The particles must be fully suspended during use to assure proper binding.

Mag-Bind® Blood & Tissue DNA 96 Kit - Blood (250µL) Protocol

The procedure below has been optimized for use with 250 µL FRESH or FROZEN blood samples. Buffy coat can also be used.

Materials and Reagents to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Nuclease-free water
- Magnetic separation device (Recommend AlpAqua Magnum™ EX, Part#A000380)
- Vortexer
- 96-well Microplate (500 µL) (Cat# EZ9604) or desired elution plate
- 2 mL 96-well deep-well plates (Recommend Nunc, Part#278752) or desired plate compatible with the magnetic separation device
- Multichannel pipettes and reagent reservoirs
- Sealing film (Cat# AC1200)
- Optional: RNase A (10 mg/mL)
- Optional: PBS
- Heat block, incubator, or water bath capable of 70°C

Before Starting:

- Prepare SPM Wash Buffer, HDQ Binding Buffer, and VHB Buffer according to the "Preparing Reagents" section on Page 4.

1. Add blood samples to a 96-well deep-well plate (2 mL). Bring the volume up to 250 µL with PBS (not provided) or Elution Buffer (provided with this kit) if volume of blood is less than 250 µL.

2. Add 20 µL Proteinase K Solution to each sample. Immediately proceed to the next step.

Optional: Add 5 µL RNase A to each sample. Vortex or pipet up and down 20 times to mix.

3. Add 290 µL AL Buffer to each sample. Vortex at maximum speed or pipet up and down 20 times. Proper mixing is crucial for good yield.
   Note: For automated protocols tip mix yields best results and is recommended.

4. Incubate at 70°C for 10 minutes.

5. Add 400 µL HDQ Binding Buffer and 20 µL Mag-Bind® Particles HDQ to each sample. Vortex at maximum speed for 10 minutes.

   Note: HDQ Binding Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions. HDQ Binding Buffer and Mag-Bind® Particles HDQ can be prepared as a mastermix. Mix only what is needed for each run.

6. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

7. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

8. Remove the plate from the magnetic separation device.

9. Add 600 µL VHB Buffer to each sample.

   Note: VHB Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

10. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 20 times or vortexing for 1 minute.
    Note: Complete resuspension of the Mag-Bind® Particles HDQ is critical for obtaining good purity.

11. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

12. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

13. Remove the plate from the magnetic separation device.

14. Repeat Steps 9-13 for a second VHB Buffer wash step.

15. Add 600 µL SPM Wash Buffer to each sample.
Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

16. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 20 times or vortexing for 1 minute.

17. Let sit at room temperature for 1 minute.

18. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

19. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

20. Leave the plate on the magnetic separation device. Add 500 µL nuclease-free water and immediately aspirate. Do not leave nuclease-free water on Mag-Bind® Particles HDQ for more than 60 seconds.

21. Remove the plate from the magnetic separation device.

22. Add 50-200 µL Elution Buffer to elute DNA from the Mag-Bind® Particles HDQ. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 50 times.
Note: Heat Elution Buffer or nuclease-free water to 70°C to improve yield.

23. Let sit at room temperature for 5 minutes.

24. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

25. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

Mag-Bind® Blood & Tissue DNA 96 Kit - Tissue Protocol

This method allows genomic DNA isolation from up to 10 mg tissue. Yields will vary depending on the source.

Materials and Equipment to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Nuclease-free water
- Magnetic separation device (Recommend AlpAqua Magnum™ EX, Part#A000380)
- Vortexer
- Centrifuge with swing-bucket rotor capable of 4,000 x *g*
- Centrifuge adaptor for 96-well plates
- Shaking water bath capable of 55°C
- 96-well Microplate (500 µL) (Cat# EZ9604) or desired elution plate
- 2 mL 96-well deep-well plates (Recommend Nunc, Part#278752) or desired plate compatible with the magnetic separation device
- Multichannel pipettes and reagent reserviors
- Sealing film (Cat# AC1200)
- Optional: RNase A (10 mg/mL)
- Optional: PBS
- Optional: Heat block, incubator, or water bath capable of 70°C
- Optional: Liquid nitrogen and mortar and pestle

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Set water baths, incubators, or heat blocks to 55°C and 70°C.

OPTIONAL: Although mechanical homogenization of tissue is not necessary, pulverizing the samples in liquid nitrogen will improve lysis and reduce incubation time. Once the liquid nitrogen has evaporated, transfer the powdered tissue to a clean 96-well deep-well plate. Add 250 µL TL Buffer and proceed to Step 3 below.

1. Mince up to 10 mg tissue and transfer to a 96-well deep-well plate.

   Note: Cutting the tissue into small pieces can speed up lysis.

2. Add 250 µL TL Buffer.

3. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.

4. Incubate at 55°C in a shaking water bath.

   Note: If a shaking water bath is not available, vortex the sample every 20-30 minutes. Lysis time depends on amount and type of tissue, but is usually under 3 hours. The lysis can proceed overnight.

   Important: Some tissue may contain material that can not be digested with proteinase; centrifuge the plate at maximum speed for 5 minutes to remove the undigested material. Transfer the cleared lysate to a new plate.

OPTIONAL: Certain tissues such as liver have high levels of RNA which will be purified with DNA using this kit. While it will not interfere with PCR, the RNA can be removed at this point. Add 5 µL RNase A (assuming a sample size of 10 mg) and let sit at room temperature for 2 minutes. Proceed to Step 5.

5. Centrifuge at maximum speed for 5 minutes to pellet undigested tissue debris and hair.

6. Carefully transfer 200 µL of the supernatant to a new 96-well deep-well plate without disturbing the undigested pellet.

7. Add 230 µL AL Buffer to each sample. Vortex at maximum speed for 10 minutes or pipet up and down 20 times. Proper mixing is crucial for good yield.
   Note: For automated protocols tip mix yields best results and is recommended.

8. Add 320 µL HDQ Binding Buffer and 20 µL Mag-Bind® Particles HDQ to each sample. Vortex at maximum speed for 10 minutes.

   Note: HDQ Binding Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions. HDQ Binding Buffer and Mag-Bind® Particles HDQ can be prepared as a mastermix. Mix only what is needed.

9. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

10. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

11. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

12. Add 600 µL VHB Buffer to each sample.

    Note: VHB Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

13. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 20 times or vortexing for 1 minute.

    Note: Complete resuspension of the Mag-Bind® Particles HDQ is critical for obtaining good purity.

14. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

15. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

16. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

17. Repeat Steps 12-16 for a second VHB Buffer wash step.

18. Add 600 µL SPM Wash Buffer to each sample.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

19. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 20 times or vortexing for 1 minute.

20. Let sit at room temperature for 1 minute.

21. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

22. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

23. Leave the plate on the magnetic separation device. Add 500 µL nuclease-free water and immediately aspirate. Do not leave nuclease-free water on Mag-Bind® Particles HDQ for more than 60 seconds.

24. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

25. Add 100-200 µL Elution Buffer or nuclease-free water to elute DNA from the Mag-Bind® Particles HDQ. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 50 times.

    Note: Heat Elution Buffer or nuclease-free water to 70°C to improve yield.

26. Let sit at room temperature for 5 minutes.

27. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

28. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

Mag-Bind® Blood & Tissue DNA 96 Kit - Cultured Cells Protocol

This protocol is designed for rapid isolation of up to 25 µg genomic DNA from up to 5 x 10^6 cultured cells.

Materials and Equipment to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Nuclease-free water
- Magnetic separation device (Recommend AlpAqua Magnum™ EX, Part#A000380)
- Vortexer
- Shaking water bath capable of 55°C
- 96-well Microplate (500 µL) (Cat# EZ9604) or desired elution plate
- 2 mL 96-well deep-well plates (Recommend Nunc, Part#278752) or desired plate compatible with the magnetic separation device
- Multichannel pipettes and reagent reserviors
- Sealing film (Cat# AC1200)
- Cold PBS (4°C)
- Optional: RNase A (10 mg/mL)
- Optional: Heat block, incubator, or water bath capable of 70°C

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Set shaking water bath to 55°C.

1. Prepare the cell suspension.

   1a. Frozen cell samples should be thawed before starting this protocol. Pellet cells by centrifugation. Wash the cells with cold PBS (4°C) and resuspend cells in 180 µL cold PBS. Proceed with Step 2 of this protocol.

   1b. For cells grown in suspension, pellet 5 x 10^6 cells at 1,200 x *g* in a centrifuge tube. Discard the supernatant, wash the cells once with cold PBS (4°C), and resuspend cells in 180 µL cold PBS. Proceed with Step 2 of this protocol.

   1c. For cells grown in a monolayer, harvest the cells by either using a trypsin treatment or cell scraper. Wash cells twice in cold PBS (4°C) and resuspend the cells with 180 µL cold PBS. Proceed with Step 2 of this protocol.

2. Add 20 µL Proteinase K Solution. Immediately proceed to the next step.

3. Add 230 µL AL Buffer to each sample. Vortex at maximum speed for 10 minutes. Proper mixing is crucial for good yield.
   Note: For automated protocols tip mix yields best results and is recommended.

4. Incubate at 55°C in a shaking water bath for 10 minutes.

   Note: If a shaking water bath is not available, vortex the sample every 2-3 minutes.

5. Transfer the samples into a 96-well deep-well plate.

Optional: Add 5 µL RNase A to each sample. Vortex or pipet up and down 20 times to mix.

6. Add 320 µL HDQ Binding Buffer and 20 µL Mag-Bind® Particles HDQ to each sample. Vortex at maximum speed for 10 minutes.

   Note: HDQ Binding Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions. HDQ Binding Buffer and Mag-Bind® Particles HDQ can be prepared as a mastermix. Mix only what is needed.

7. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

8. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

9. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

10. Add 600 µL VHB Buffer to each sample.

    Note: VHB Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

11. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 20 times or vortexing for 1 minute.

    Note: Complete resuspension of the Mag-Bind® Particles HDQ is critical for obtaining good purity.

12. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

13. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

14. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

15. Repeat Steps 10-14 for a second VHB Buffer wash step.

16. Add 600 µL SPM Wash Buffer to each sample.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

17. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 20 times or vortexing for 1 minute.

18. Let sit at room temperature for 1 minute.

19. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

20. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

21. Leave the plate on the magnetic separation device. Add 500 µL nuclease-free water and immediately aspirate. Do not leave nuclease-free water on Mag-Bind® Particles HDQ for more than 60 seconds.

22. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

23. Add 100-200 µL Elution Buffer or nuclease-free water to elute DNA from the Mag-Bind® Particles HDQ. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 50 times.

    Note: Heat Elution Buffer or nuclease-free water to 70°C to improve yield.

24. Let sit at room temperature for 5 minutes.

25. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

26. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

Mag-Bind® Blood & Tissue DNA 96 Kit - Saliva Protocol

Materials and Equipment to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Nuclease-free water
- Magnetic separation device (Recommend AlpAqua Magnum™ EX, Part#A000380)
- Vortexer
- 96-well Microplate (500 µL) (Cat# EZ9604) or desired elution plate
- 2 mL 96-well deep-well plates (Recommend Nunc, Part#278752) or desired plate compatible with the magnetic separation device
- Multichannel pipettes and reagent reserviors
- Sealing film (Cat# AC1200)
- Optional: RNase A (10 mg/mL)
- Optional: Heat block, incubator, or water bath capable of 70°C

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Set shaking water bath to 55°C.

1. Transfer 250 µLstabilized saliva samples (e.g. DNA Genotek Oragene®, Mawi iSWAB™, Biomatrica® DNAgard® Saliva) into a 96-well deep-well plate.

2. Add 20 µL Proteinase K Solution.

Optional: Add 5 µL RNase A to each sample. Vortex or pipet up and down 20 times to mix.

3. Add 290 µL AL Buffer to each sample. Vortex at maximum speed for 10 minutes or pipet up and down 20 times. Proper mixing is crucial for good yield.

Note: Tip mixing is recommended for automated protocols.

4. Add 400 µL HDQ Binding Buffer and 20 µL Mag-Bind® Particles HDQ to each sample. Vortex at maximum speed for 10 minutes.

   Note: HDQ Binding Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions. HDQ Binding Buffer and Mag-Bind® Particles HDQ can be prepared as a mastermix. Mix only what is needed.

5. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

6. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

7. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

8. Add 600 µL VHB Buffer to each sample.

   Note: VHB Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

9. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 20 times or vortexing for 1 minute.

   Note: Complete resuspension of the Mag-Bind® Particles HDQ is critical for obtaining good purity.

10. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

11. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

12. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

13. Repeat Steps 8-12 for a second VHB Buffer wash step.

14. Add 600 µL SPM Wash Buffer to each sample.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

15. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 20 times or vortexing for 1 minute.

16. Let sit at room temperature for 1 minute.

17. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

18. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

19. Leave the plate on the magnetic separation device. Add 500 µL nuclease-free water and immediately aspirate. Do not leave nuclease-free water on Mag-Bind® Particles HDQ for more than 60 seconds.

20. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

21. Add 100-200 µL Elution Buffer or nuclease-free water to elute DNA from the Mag-Bind® Particles HDQ. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 50 times.

    Note: Heat Elution Buffer or nuclease-free water to 70°C to improve yield.

22. Let sit at room temperature for 5 minutes.

23. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

24. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

Mag-Bind® Blood & Tissue DNA 96 Kit - Buccal Swabs Protocol

Materials and Equipment to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Nuclease-free water
- Magnetic separation device (Recommend AlpAqua Magnum™ EX, Part#A000380)
- Vortexer
- Centrifuge with swing-bucket rotor capable of 4,000 x *g*
- Centrifuge adaptor for 96-well plates
- Shaking water bath capable of 55°C
- 96-well Microplate (500 µL) (Cat# EZ9604) or desired elution plate
- 2 mL 96-well deep-well plates (Recommend Nunc, Part#278752) or desired plate compatible with the magnetic separation device
- Multichannel pipettes and reagent reservoirs
- Sealing film (Cat# AC1200)
- Optional: RNase A (10 mg/mL)
- Optional: Heat block, incubator, or water bath capable of 70°C

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Set shaking water bath to 55°C.

1. Cut off the buccal brush or swab head and place each swab into a well of a 96-well deep-well plate.

2. Add 290 µL AL Buffer and 250 µL Elution Buffer.

   Note: AL Buffer and Elution Buffer can be prepared as a mastermix.

3. Add 20 µL Proteinase K Solution.

4. Incubate at 55°C in a shaking water bath for 10 minutes.

   Note: If a shaking water bath is not available, vortex the plate every 5-10 minutes.

5. Centrifuge at 3000 x *g* for 2 minutes.

6. Transfer 500 µL lysate into a new 96-well deep-well plate. Do not transfer the swabs to the new plate.

Optional: Add 5 µL RNase A to each sample. Vortex or pipet up and down 20 times to mix.

7. Add 350 µL HDQ Binding Buffer and 20 µL Mag-Bind® Particles HDQ to each sample. Vortex at maximum speed for 10 minutes.

   Note: HDQ Binding Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions. HDQ Binding Buffer and Mag-Bind® Particles HDQ can be prepared as a mastermix. Mix only what is needed.

8. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

9. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

10. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

11. Add 600 µL VHB Buffer to each sample.

    Note: VHB Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

12. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 20 times or vortexing for 1 minute.

    Note: Complete resuspension of the Mag-Bind® Particles HDQ is critical for obtaining good purity.

13. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

14. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

15. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

16. Repeat Steps 11-15 for a second VHB Buffer wash step.

17. Add 600 µL SPM Wash Buffer to each sample.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

18. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 20 times or vortexing for 1 minute.

19. Let sit at room temperature for 1 minute.

20. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

21. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

22. Leave the plate on the magnetic separation device. Add 500 µL nuclease-free water and immediately aspirate. Do not leave nuclease-free water on Mag-Bind® Particles HDQ for more than 60 seconds.

23. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

24. Add 100-200 µL Elution Buffer or nuclease-free water to elute DNA from the Mag-Bind® Particles HDQ. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 50 times.

    Note: Heat Elution Buffer or nuclease-free water to 70°C to improve yield.

25. Let sit at room temperature for 5 minutes.

26. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

27. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

Mag-Bind® Blood & Tissue DNA 96 Kit - Mouse Tail Snips Protocol

Materials and Equipment to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Nuclease-free water
- Magnetic separation device (Recommend AlpAqua Magnum™ EX, Part#A000380)
- Vortexer
- Centrifuge with swing-bucket rotor capable of 4,000 x *g*
- Centrifuge adaptor for 96-well plates
- Shaking water bath capable of 55°C
- 96-well Microplate (500 µL) (Cat# EZ9604) or desired elution plate
- 2 mL 96-well deep-well plates (Recommend Nunc, Part#278752) or desired plate compatible with the magnetic separation device
- Multichannel pipettes and reagent reserviors
- Sealing film (Cat# AC1200)
- Optional: RNase A (10 mg/mL)
- Optional: Heat block, incubator, or water bath capable of 70°C

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Set water bath to 55°C.

1. Snip a 2-5 mm piece of mouse tail, cut into several pieces, and transfer the pieces to a 96-well deep-well plate.

   Note: Follow all regulations regarding the safe and humane treatment of animals. Mice should not be older than 6 weeks since lysis will be more difficult in older animals resulting in suboptimal DNA yields. If possible, obtain tail biopsies at 2-4 weeks and freeze samples at -70°C until DNA is extracted.

2. Add 250 µL TL Buffer.

3. Add 20 µL of Proteinase K Solution. Vortex to mix thoroughly.

4. Incubate the plate at 55°C in a shaking water bath for 1-4 hours or until lysis is complete.

   Note: If a shaking water bath is not available, vortex the samples vigorously every 20-30 minutes. Incomplete lysis may significantly reduce DNA yields. Incubation time for complete tail lysis is dependent on length of tail snip and age of animal, e.g. a 5 mm tail piece from a 2 week old mouse typically will lyse in 2 hours. For older animals, an overnight incubation may improve yields. Note that bone and hair will not lyse.

5. Centrifuge at maximum speed for 5 minutes to pellet undigested tissue debris and hair.

6. Carefully transfer 200 µL of the supernatant to a new 96-well deep-well plate without disturbing the undigested pellet.

OPTIONAL: Mouse tail tissue contains RNA that can purify with the DNA. This will not interfere with PCR reactions, but other enzymatic reactions may be affected. To remove RNA, add 5 µL RNase A and let sit at room temperature for 2 minutes.

7. Add 230 µL AL Buffer to each sample. Vortex at maximum speed for 10 minutes. or pipet up and down 10 times. Proper mixing is crucial for good yield.

Note: Tip mixing is recommended for automated protocols.

8. Add 320 µL HDQ Binding Buffer and 20 µL Mag-Bind® Particles HDQ to each sample. Vortex at maximum speed for 10 minutes.

   Note: HDQ Binding Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions.

   Note: HDQ Binding Buffer and Mag-Bind® Particles HDQ can be prepared as a mastermix. Mix only what is needed.

9. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

10. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

11. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

12. Add 600 µL VHB Buffer to each sample.

    Note: VHB Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

13. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 20 times or vortexing for 1 minute.

    Note: Complete resuspension of the Mag-Bind® Particles HDQ is critical for obtaining good purity.

14. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

15. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

16. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

17. Repeat Steps 12-16 for a second VHB Buffer wash step.

18. Add 600 µL SPM Wash Buffer to each sample.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

19. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 20 times or vortexing for 1 minute.

20. Let sit at room temperature for 1 minute.

21. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

22. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

23. Leave the plate on the magnetic separation device. Add 500 µL nuclease-free water and immediately aspirate. Do not leave nuclease-free water on Mag-Bind® Particles HDQ for more than 60 seconds.

24. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

25. Add 100-200 µL Elution Buffer or nuclease-free water to elute DNA from the Mag-Bind® Particles HDQ. Resuspend the Mag-Bind® Particles HDQ by pipetting up and down 50 times.

    Note: Heat Elution Buffer or nuclease-free water to 70°C to improve yield.

26. Let sit at room temperature for 5 minutes.

27. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

28. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **(800-832-8896)**.

Problem	Cause	Solution
Low DNA yield	Incomplete resuspension of Mag-Bind® Particles HDQ	Resuspend the Mag-Bind® Particles HDQ by vortexing vigorously before use
	Frozen blood samples not mixed properly after thawing	Thaw the frozen blood at room temperature and gently mix the blood by inverting
	Inefficient cell lysis due to inefficient mix of AL Buffer and sample	Make sure the sample is thoroughly mixed with AL Buffer.
	Loss of Mag-Bind® Particles HDQ during operation	Avoid disturbing the Mag-Bind® Particles HDQ during aspiration
	DNA remains bound to Mag-Bind® Particles HDQ	Increase elution volume and incubate at for 15 minutes; pipet up and down 50 to 100 times
	DNA washed off	Dilute SPM Wash Buffer by adding appropriate volume of ethanol prior to use (see Page 4 for instructions)
	Ethanol is not added into VHB buffer	Make sure to add ethanol to the VHB Buffer (see Page 4 for instructions)
Mag-Bind® Particles HDQ do not completely clear from solution	Too short of magnetizing time	Increase collection time on the magnet
Gel-like material in the eluted DNA	Blood is too old	Remove the gel-like material by centrifugation; recommend using fresh blood
		Use 8 mM NaOH as elution buffer
Problems in downstream applications	Salt carry-over	SPM Wash Buffer must be at room temperature
	Ethanol carry-over	Dry the Mag-Bind® Particles HDQ before elution

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Elution Buffer (EB Buffer), 100 mL	PDR048
Elution Buffer (EB Buffer), 500 mL	PD089
RNase A, 400 µL	AC117
RNase A, 5 mL	AC118
E-Z 96 Homogenizer Plate (1)	HCR9601-01
E-Z 96 Homogenizer Plate (4)	HCR9601-02
1.5 mL DNase/RNase-free Microcentrifuge Tubes	SSI-1210-00
2 mL DNase/RNase-free Microcentrifuge Tubes	SSI-1310-00
Sealing Film	AC1200

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